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SHORT COMMUNICATION

THE PREPARATION OF L- AND D-α-AMINO-β-METHYLAMINOPROPIONIC ACIDS AND THE IDENTIFICATION OF THE COMPOUND ISOLATED FROM *CYCAS CIRCINALIS* AS THE L-ISOMER

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Abstract—An improved method for the synthesis of DL-α-acetamino-β-methylaminopropionic acid and the use of this compound for the synthesis of the two optical isomers of α-amino-β-methylaminopropionic acid are described. The amino acid previously isolated from seeds of *Cycas circinalis* has been identified as the L-isomer. Unlike the natural amino acid and the synthetic L-isomer, the D-isomer failed to produce toxic effects when injected in comparable amounts into chicks and young rats.

INTRODUCTION AND DISCUSSION

THE ISOLATION of α-amino-β-methylaminopropionic acid from seeds of *Cycas circinalis* and its synthesis have been described.[1] Both the natural and synthetic compounds have been shown to be toxic to rats and chicks.[2, 3]

In order to establish the configuration of the natural compound and to determine the relative toxicities of the two optical isomers it was necessary to synthesize both. This has been done by modifying the original procedure for the synthesis of DL-α-acetamino-β-methylaminopropionic acid[4] to give greatly improved yields and then using this compound for the synthesis of both isomers. The racemic *N*-acetyl derivative was incubated with an L-amino acid acylase[5] to liberate L-α-amino-β-methylaminopropionic acid. This compound was removed by ion-exchange chromatography,[6] and the residual D-acetyl derivative hydrolysed with strong acid to give the corresponding D-α-amino-β-methylaminopropionic acid. To obtain complete hydrolysis of the L-α-acetamino-β-methylaminopropionic acid it was found necessary to incubate the racemic mixture with the enzyme twice, removing the free amino acid between incubations. The yield of L-α-amino-β-methylaminopropionic acid obtained by enzymatic hydrolysis was affected by the pH of the incubation mixture; loss of product (due apparently to carboxylase activity of the enzyme preparation) being most

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noticeable when the pH of the mixture fell to 6·5 or below. Total yield of the separate isomers obtained in the manner described was consistently lower than the yield of the DL-α-amino-β-methylaminopropionic acid prepared by direct acid hydrolysis of DL-α-acetamino-β-methylaminopropionic acid.

The specific rotation of the synthetic L-amino acid was found to be the same as that of the amino acid isolated from *C. circinalis*.

Earlier experiments showed that the natural α-amino-β-methylaminopropionic acid (shown in this paper to be the L-isomer) was more toxic to chicks than the racemic compound,[2] which suggested that the D-isomer was less toxic than the L-isomer. This point has been re-examined now that both isomers are available and the original deduction confirmed.

EXPERIMENTAL AND RESULTS

Synthesis of DL-α-Acetamino-β-*Methylaminopropionic Acid*

α-Acetaminoacrylic acid (20 g) was dissolved in 100 ml of a solution of approximately 30 per cent aqueous methylamine and allowed to stand for 72 hr at 40°. Excess methylamine was removed by concentrating the reaction mixture to a syrup in a rotary evaporator under reduced pressure maintaining the temperature below 40°. On adding ethanol to the syrup, colourless crystals separated. After filtration, the crystals were washed with a further small volume of ethanol, dissolved in the minimum volume of methanol and reprecipitated by the gradual addition of dry diethyl ether.

Yield: 17·8 g (71 per cent of theoretical, cf. lit.[4] 24 per cent), m.p., 162–163°. (Found: C, 45·45; H, 7·79; N, 16·95. Calc. for $C_6H_{12}O_3N_2$: C, 45·00; H, 7·50; N, 17·50 per cent.)

Synthesis of DL-α-*Amino-β-Methylaminopropionic Acid*

DL-α-Acetamino-β-methylaminopropionic acid (4 g) was refluxed for 2 hr with 2·5 N HCl (80 ml), and the solution concentrated to a syrup under reduced pressure. The residue was redissolved in water and reconcentrated several times to remove HCl. When free of excess acid the solution was treated with charcoal, filtered, concentrated and the colourless residue recrystallized from aqueous ethanol as the amino acid hydrochloride.

Yield: 2·7 g (71 per cent of the theoretical), m.p., 165–167°. Apparent p*K* values, 2·1, 6·5 and 9·8.

Synthesis of L-α-*Amino-β-Methylaminopropionic Acid*

DL-α-Acetamino-β-methylaminopropionic acid (4 g) was dissolved in water (250 ml) and the pH adjusted to 7·6 with 2 N LiOH. The enzyme [200 mg of Acylase I from hog kidney (Sigma Chemical Company)] was added and the mixture incubated for 30 hr at 37°. The pH of the mixture was then adjusted to 5·0 (the isoelectric point of Acylase I) with acetic acid and after standing for 17 hr at 4° charcoal was added and the precipitate removed by filtration. A sample of the filtrate was subjected to high-voltage ionophoresis at pH 3·6 on paper and it was found that less than one-half of the original DL-α-acetamino-β-methylaminopropionic acid had been hydrolysed during incubation. The filtrate then was passed through a column (75 × 2·5 cm) of a weakly acidic cation exchange resin [Zeo-Karb 226 (52–100 mesh)] in the H^+ form. The column was washed with water and the effluent collected in 10-ml fractions. The unhydrolysed acetyl derivative was contained in fractions 36–119 while the free acid was retained on the column. The fractions containing the unhydrolysed *N*-acetyl derivative were combined and the pH adjusted to 7·6. After adding a further 200 mg of enzyme the combined fractions were again incubated for 30 hr at 37°. The enzyme was removed as before and on ionophoresis a sample of the filtrate was found to contain free α-amino-β-methylaminopropionic acid confirming that hydrolysis of L-α-acetamino-β-methylaminopropionic acid had not been complete after the first incubation. The filtrate was again passed through the cation exchange column and a 10 ml fraction of the effluent containing unchanged *N*-acetyl derivative was adjusted to pH 7·6 and subjected to further incubation with fresh enzyme. No further free amino acid was detected after this third incubation, indicating that enzymatic hydrolysis had been complete at the end of the second large-scale incubation. Complete hydrolysis of L-α-acetamino-β-methylaminopropionic acid in a single incubation was not achieved by prolonging the period of incubation, by adjusting the pH at intervals during the incubation, or by adding fresh enzyme to the reaction mixture. These findings suggest that the enzyme may be inhibited by the one or both of the end-products. After the unhydrolysed *N*-acetyl derivative had been removed, the column was washed with a further 2 l. of water and the free amino acid eluted with 0·3 N HCl. The fractions containing the free amino acid were combined (900 ml) and evaporated in a rotary evaporator at reduced pressure. The residue was freed of HCl, decolorized and recrystallized from aqueous ethanol as before to give L-α-amino-β-methylaminopropionic acid hydrochloride.

Yield 0·96 g (49·4 per cent of theoretical), m.p. 168°

$$[\alpha]_D^{20} + 35° \text{ (C 1·15, 5 N HCl)}$$

cf. $[\alpha]_D^{20} + 35°$ (C 1·15, 5 N HCl) for the hydrochloride of the natural compound isolated from *Cycas circinalis*.

Synthesis of D-α-*Amino-β-Methylaminopropionic Acid*

After incubating DL-α-acetamino-β-methylaminopropionic acid twice with Acylase I and removing L-α-amino-β-methylaminopropionic acid as described; those fractions of effluent from the cation exchange column containing D-α-acetamino-β-methylaminopropionic acid were combined (1800 ml) and evaporated to dryness under reduced pressure. The residue was dissolved in 2·5 N HCl (40 ml) and refluxed for 2 hr. The solution was then concentrated, freed of excess HCl, decolorized, concentrated once more and the residue recrystallized from aqueous ethanol to give D-α-amino-β-methylaminopropionic acid hydrochloride.

Yield: 1·05 g (54·4 per cent of theoretical), m.p. 168°

$$[\alpha]_D^{20} - 35° \text{ (C 1·2, 5 N HCl)}$$

The Relative Toxicities of L- *and* D-α-*Amino-β-Methylaminopropionic Acids*

Chicks used were 7 days post-hatch, strain R-X-S, weight 40–45 g, male. The rats were Wistar strain, weight 45–60 g female. Solutions of D- and L-α-amino-β-methylaminopropionic acid were adjusted to pH 6·5 with NaOH before intraperitoneal injection.

A group of three chicks was injected with the L-isomer (3–7 μmoles/g body wt.) another group with the same quantity of the D-isomer and a third group with 0·4 ml of 0·9 per cent saline solution. Those treated with the L-isomer developed characteristic weakness followed by convulsions and head retractions and eventually recovered.[2] No unusual symptoms were noted in the groups injected with the D-isomer or with the saline solution.

The experiment was repeated with groups of three rats injecting 6–14 μmoles/g body wt. Those animals injected with the L-isomer developed a dragging gait, weakness and convulsions. Again no such symptoms were noted in the group treated with the D-isomer.